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Original article

Role of toll like receptors in bacterial and viral diseases – A systemic approach

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ABSTRACT

Background: Toll like receptors are key-receptors of the innate immune system, but their role against bacterial and viral infections are yet to be understood.
Aim: The present study is aimed to investigate the diversity and frequency distribution of 10 TLR genes among typhoid fever and HIV+ patients. In this study, 44 samples were taken from typhoid patients and 55 samples from HIV+ patients.
Patients and methods: Widal test positive samples (>1:80) in case of typhoid and the percentage of CD4+ count in case of HIV+ patient were considered for the PCR-SSP analysis.
Results: We found that the frequencies of TLR1 and TLR6 were highest in typhoid patients, whereas the frequencies of TLR8 and TLR9 displayed higher among HIV+ patients. Chi-square values were significant for TLR8 and TLR10 in the case of typhoid patients, whereas in HIV patients significant values were considered for TLR2, TLR4, TLR8 and TLR9 respectively. The odds ratio calculated highest for TLR1 and TLR6 among typhoid patients. TLR4 and TLR9 calculated were highest odd for HIV+ patients. A door line association of TLRs with the disease was found when the relative risk was calculated for TLR2 (1.72), TLR3 (1.21) and TLR10 (1.98) in bacterial infection, whereas in case of viral infection relative risk was calculated for TLR4 (1.62), TLR8 (1.18) and in TLR9 (1.16).
Conclusion: This study reports the frequency distribution and association of human TLR genes with the bacterial and viral infection in the North Bengal region of India for the first time. It also signified the gene- disease- environment association study in case of infectious diseases and also the risk factors of bacterial and viral infections in this region. It also depicts the role of TLRs in the recognition of the pathogens.

1. Introduction

Free-living organisms have the ability to cope up with the new environment by modifying their gene expression patterns [1]. Extensive variations at the genomic level made the analyses of gene-disease association and their susceptibility possible [2]. The frequency of genes and their alleles vary between different populations in case of different diseases [3]. Till date, slow progress has been observed in the field of genome-wide association studies for the infectious disease in comparison to other diseases. However, some studies involving bacterial and viral diseases contribute substantially to the growing knowledge of the host genetic variations and treatment of the diseases [4,1]. Toll like receptors that regulate both innate and adaptive immune response and polymorphism in the TLR genes has been investigated in case of various diseases [5].

Enteric fever has become an alarming infection nowadays among populations in some areas of India [6]. *Salmonella enterica* serotype *typhi* (S. typhi) is a gram-negative bacterium, restricted in human and cause a wide range of food- and water-borne diseases ranging from self-limiting gastroenteritis to systemic typhoid fever [7]. The occurrence of typhoid fever is less in developing and industrialized countries, but high in the countries of South-East Asia including India [6]. According to Crump et al. (2004) typhoid fever caused over 20 million illnesses and over 200 thousand deaths during the year 2000 [8]. Poor sanitation, lack of safe drinking water supply and low socioeconomic conditions

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has amplified the disease in India, which increases the morbidity and mortality [9].

Primarily, TLR4 and TLR5 play major roles in the activation of immune responses against LPS and flagellin. TLR4 polymorphisms among the Asian Malay population confer a higher risk for typhoid infection in case of *S. typhi* [10]. Genetic association study among the Vietnam population has proven no association of TLR5392STOP stop codon with typhoid fever [11]. Binding site modulation of TLR gene receptors against the lipopolysaccharide (LPS), flagellin or other antigens of *Salmonella typhi* evokes the host immune response during typhoid fever [12].

On the other side susceptibility to the human immunodeficiency virus (HIV) infection and disease progression are variable among populations [13]. A small percentage of 0.2% of the HIV-1 sero-positive patient is able to control the HIV-1 infection over 10 years. The adult HIV prevalence at national level has 0.26% in 2015 [14]. It means that they can maintain a viral load of fewer than 50 copies of HIV-1 RNA per ml [15] more of HIV-1 RNA will accelerate the prevalence of the disease. Infection with human immunodeficiency virus (HIV) results in progressive deterioration of the immune system in untreated patients [16]. Different TLRs expressed on different cell types in the human immune system and up-regulated by the effect of cytokines like IFN-y induces the expression of TLR4 in peripheral blood monocytes [17].

The HIV disease progression can be estimated by measuring marker expression in the course of the disease. The degree of CD4+ T-cell depletion is the most important marker for the detection of HIV [18]. Indeed, the most characteristic feature of HIV is the depletion of the CD4+ T-helper-inducer subset of T cells. The other markers that are also reliable for estimating HIV disease progression include b2m, neopterin, IgG, IgM, anti-p24, anti-gp120, TNF etc. [18].

Several association studies have been reported in case of TLRs with HIV. It has been reported that depletion of CD4+(Th2) cells in HIV positive individuals releases bacterial components that directly activates TLR4 [19]. According to Baenziger et al. (2009), the chronic activation of TLR7 leads to immune dysregulation in murine model which is similar to human [20]. Several other TLRs are also associated with HIV disease progression.

2. Subjects and methods

2.1. Selection of patients for typhoid fever

Typhoid patients were diagnosed by expert doctors of North Bengal Medical College and Hospital, Shushrutnagar, Siliguri (latitude & longitude 26.7271°N, 88.3953°E) on the basis of specific symptoms of typhoid fever. Screening of the typhoid patients were based on the positive results of the Widal test [21]. The serum agglutination test was done against S. typhi "O" and "H" antigens using a Salmonella antigen kit (Beacon diagnostic Pvt. Ltd, Navsari India). The test was performed according to the manufacturer's instruction. The serum antibody titer of 1:80 or above was considered positive for the typhoid fever.

2.2. Selection of patients for human immunodeficiency virus

Fifty-five HIV-infected patients (including 33 women, 22 men and, median age-34) and 70 healthy individuals (47 women, 23 men, a range of 20–52) were included in this study (Table 1). Individuals under any sorts of medication were excluded from the control group (n = 70) in our study. Positive HIV patients were selected based on the viral infection and counting of CD4+ cells within the range of 156–756 $\times$ 10^6 cells/L. Laboratory values for patients who did not receive anti retroviral therapy (ART) had 117–730 $\times$ 10^6 cells/L CD4+ cells per litre, but CD4+ count became 142–890 $\times$ 10^6 cells/L after receiving ART.

Table 1
Demographic characteristics of Typhoid fever patients and HIV+ patients and Healthy donors.

Sex	Typhoid patients	Healthy Donors
Male	18 (40%)	27 (38%)
Female	26 (60%)	43 (62%)
Total	**44**	**70**
Sex	HIV+ patients	Healthy Donors
Male	22(40%)	23(32%)
Female	33(60%)	47(67%)
Total	**55**	**70**

HIV-Human Immunodeficiency Virus.

2.3. Sample collection

3 mL of venous blood was collected from Forty-four typhoid patients between December 2014 to June 2016 from Siliguri and adjoining areas of West Bengal and Seventy healthy control subjects were taken after screening by the doctors. A detailed clinical report was taken from the patients who were admitted to the hospitals and primary health care of Siliguri and adjacent areas with gastro-intestinal problems.

The demographic characteristics of Fifty-five HIV+ patient and seventy healthy donors are represented in Table 1. 5 mL of blood samples were collected from each individual who attended the District Hospital with prior informed consent. Simultaneously, samples were collected from healthy donors after proper examination by the doctors.

The samples were stored in EDTA at −20 °C until use. The investigation was approved by the Human ethics committee of the Department of Zoology, University of North Bengal (Zoo/4133/2011) and performed in accordance with the Declaration of Helsinki, 1975.

2.4. DNA extraction and PCR-SSP typing

Genomic DNA was extracted from the blood samples using the standard Phenol-Chloroform extraction method with slight modifications. DNA integrity was checked in UV-transilluminator. O.D value was taken with 260/280 nm. Value of 1 or above was found as good quality of the DNA (Fig 1a). PCR-SSP typing was done for all the 10 TLRs. The TLR primers were designed using NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table 2) [22] and procured from the Integrated DNA Technologies, Inc, Iowa, USA. Each 25 μl PCR reaction mixture contained 5X PCR buffer (Promega Corporation, Agora, Fitchburg Center, Fitchburg, Wisconsin), 5 μL of 10 mM dNTPs, 1.5 μL of 25 mM MgCl2, 1.5 μL of primers, and 1–1.5 U of Taq DNA polymerase. 1.5–2 μl of 100 ng DNA samples were then added to the PCR mixture. The reaction conditions for PCR consisted of an initial denaturation step of 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 56.9 °C for 50 s and 72 °C for 1 min, followed by a single final extension of 72 °C for10 min. Slight modifications in the annealing temperatures of different primer sets were made as per the requirement. The PCR products were analyzed using ethidium bromide prestained 1% agarose gel electrophoresis. Samples were then visualized on UV transilluminator. All the lanes of the product loaded gel showed a control band, except for the negative control lane. The reactions were repeated to avoid false reactions where no control bands were found (Fig 1b).